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LEAD EXPOSURE AND CHILD DEVELOPMENT

An International Assessment

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Biological Monitoring of Lead Exposure in Children: Overview of Selected Biokinetic and Toxicological Issues

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SUMMARY

The biological monitoring of lead exposure in paediatric and adult human populations has usually involved one of two approaches: (1) measurement of the internal or systemic dose of lead itself in some indicator medium, or (2) quantification of some 'subcritical' effect of lead. The extent to which biological monitoring in humans accurately states both exposure risk and relative health risk remains the subject of much research. Of particular interest are (1) the biokinetic characteristics of the common indicators of exposure, (2) the development and use of kinetic models of lead metabolism, and (3) the relative merits of the use of biological effect indicators versus measurement of the toxicant in some medium.

Any successful study of the adverse effects of lead exposure in children rests heavily on the quality of the methods used to monitor the type and extent of lead exposure in these subjects, and how well such exposures can be quantitatively related to adverse health risk and population response.

In general, there are several ways to monitor the exposure of human populations to lead or other environmental pollutants. The traditional approach has been that of environmental monitoring, in which the level of toxicant is measured in those environmental media which also serve as routes of human exposure (e.g., ambient or workplace air, food, drinking water, soil). Currently, however, increasing preference is being given to biological monitoring, in which measurements are taken of the level of a pollutant, one or more of the pollutant's metabolites, or some metabolic change relatively specific for the substance, in some biological medium obtained from an exposed subject (e.g., blood, mineralizing or keratinizing tissues, excreta). Although the use of

certain biological effect indicators as exposure indices in the case of lead has been frequently recorded, this type of monitoring is more appropriately placed under the heading of health surveillance monitoring.

There are a number of recognized advantages to biological versus environmental monitoring, although the two approaches should not be viewed as being mutually exclusive. One virtue of biological monitoring is that it represents the systemic or internal level of exposure of the subject, being the result of the integration of all exposure routes and toxicokinetic parameters relating to intake and uptake of the substance.

With the increasing popularity of biological monitoring of lead exposure in children and other human populations, there is also recognition of some problems of both utility and interpretation, and these centre around the following issues: (1) the relative ease and reliability of the quantitative analysis of the toxicant; (2) the strength of the relationship between internal and external (environmental) exposure as well as interrelationships among various biological indicators of lead exposure, e.g., lead in whole blood (PbB) versus lead in teeth (PbT) or lead in hair (Pb-Hair); and (3) the relationship of the particular internal exposure measure to any quantitative health risk assessment.

Since biological monitoring is now commonly employed to assess both lead exposure and health risk relationships in young children and adult populations, and this includes the various prospective studies currently under way, it is of interest to consider some issues specific to biological monitoring of this particular toxicant. These areas of discussion include: (1) the quantitative relationship between some environmental medium and the amount present in some biological medium, e.g., the relationship of lead in air to lead in blood; (2) the state of development of various biokinetic models to provide theoretical underpinnings for lead's biokinetic behaviour in organisms; (3) the characteristics of the various methods of biological monitoring as determined by both experiment and modelling exercises, including the interrelationships among various biological indicators; (4) the quantitative relationship of exposure indices such as lead in blood to target or critical organs for lead's effects and associated dose–effect and dose–population response relationships; and (5) the relative value of lead levels in some biological medium compared to the use of certain early biological effect indicators of lead exposure. Although the quantitative relationship of lead in biological, versus lead in environmental, media is an important topic and comprises an extensive literature, this area is somewhat outside the interests of this report.

BIOKINETIC MODELLING OF THE BEHAVIOUR OF LEAD *IN VIVO*

In the broadest sense, modelling exercises use abstract and mathematical frameworks to reduce complex biological relationships into manageable and categorical descriptions. The use of models has as its purpose the rationalization of experimental information and the prediction of lead kinetics *in vivo*. Such models may be qualitative, i.e., purely descriptive in nature, or they may provide quantitative information about the discrete steps involved in the

biological handling of lead, such as the size of the transfer coefficients that govern the movement of lead among body compartments.

From the perspective of biological monitoring and the interests of the toicologist or the clinician, the relative merits of biokinetic models for lead increase with the ability of such exercises to (1) describe the actual exposure of humans and that level of apparent exposure signalled by some biological indicator; (2) provide guidance as to the best biological indicator to reflect toxicologically significant internal exposure; (3) connect biological indicator levels with amounts of toxicant in the actual target or critical organs; and finally (4) assist in the evolution of dose–effect and dose–response relationships.

In the specific case of lead metabolism, a number of models have been proposed and published over the years to rationalize the biological behaviour of lead in human subjects and experimental animals. The development and predictive utility of these models rest in large part on the considerable amount of empirical information available in the literature, relating to lead absorption, distribution, excretion, and retention in humans and test species.

The earliest models of lead toxicokinetics are typified by that of Rabinowitz *et al.* (1976, 1977), using stable lead isotope in human volunteers, and which indicate that there are at least three kinetically distinct body compartments for lead disposition *in vivo*. These compartments consist of a central blood compartment, a second lead depository in peripheral soft tissues, and, finally, the large bone compartment for lead. Lead in blood is the most kinetically labile, whereas lead in soft tissues has a somewhat larger biological half-life. The bone compartment retains lead for the longest time. Blood and soft tissues contain relatively small burdens of lead, *ca.* 1.9 and 0.6 mg respectively, while the vast majority of the body burden of lead is sequestered in a kinetically slow compartment of bone, with levels that can exceed 200 mg of the toxicant.

Kneip *et al.* (1983) developed a multi-organ compartment model based on single and chronic oral exposures of juvenile and infant baboons that allowed the estimation of different transfer coefficients for lead among body compartments in both developing and adult organisms. Using the same modelling approach and estimates for human subjects up to 20 years of age, Harley and Kneip (1984) have attempted to develop an integrated model of lead kinetics in humans of various ages. They provide estimates of organ lead levels and selected tissue lead half-lives for ages 1 through 20 in 1-year increments. Some of these estimates for selected ages are given in Tables 1 and 2.

The above modelling approaches assume well-mixed, interconnected pools under essentially steady-state conditions, and they employ first-order kinetics using coupled differential equations and linear exponential solutions. As such, the models collectively provide a reasonable description of biological disposition under rather well-defined circumstances, e.g. a low level of exposure over an extended time. In addition, the modelling approach of Harley and Kneip (1984) provides some estimation of age differences for the tissue burdens of lead in humans.

Linear models of lead biokinetics in humans and test species encounter

Table 1 Estimated biological half-life values for age-dependent tissue lead burdens[a]

	Tissue Half-Life, (days)		
Age (years)	*Bone*	*Kidney*	*Liver*
1	1135	10	23
3	1135	10	23
6	1135	10	23
8	2560	10	23
13	3421	10	23
15	3421	10	23
20	3421	10	23

[a] Adapted from Harley and Kneip, 1984

Table 2 Estimated tissue lead burdens as a function of age[a,b]

Age (years)	*Blood (μg/dl)*	*Bone (μg/g ash)*	*Kidney (μg/g wet)*
1	11.9	35.5	0.7
2	16.2	38.1	1.0
3	14.6	42.6	0.9
5	14.5	51.0	0.9
7	13.0	57.9	0.8
10	10.4	57.6	0.9
15	11.3	41.7	0.7

[a] Adapted from Harley and Kneip, 1984
[b] Based on 40% uptake/100 μg intake in males

difficulty when one must consider such phenomena as dose-dependent uptake and tissue distribution of lead and the very labile biokinetics of lead in young children. Related to the issue of dose-related biokinetics, of course, is the fact that there is a curvilinear relationship between plasma and blood lead that indicates a higher fraction of blood lead present in plasma with increasing blood lead (DeSilva, 1981; Manton and Cook, 1984).

In a series of reports, Marcus (1985a, b, c) has discussed linear and nonlinear multicompartment models of lead kinetics in mammalian systems with particular emphasis on the relationship of plasma lead to whole blood lead, a relationship which is nonlinear in nature, and the nonlinear relationship of lead in exposure media to lead in blood. Marcus (1985c) proposed four discrete pools for lead within the blood compartment: shallow and deep erythrocyte pools, diffusable lead in plasma, and protein-bound lead in plasma. In this model, Marcus employed data for a volunteer subject who ingested lead under tightly controlled conditions for a period of time (DeSilva, 1981). Different versions of the model, differing as to the mechanisms underlying the nonlinear plasma lead/whole blood lead relationship, were tested, and the one based on site-limited absorption provided the best fit for the 103 subjects studied by DeSilva (1981). The tightness of fit is particularly good at higher blood lead values, while plasma lead is underestimated at or below 30 μg Pb/dl.

Chamberlain (1985) employed a nonlinear modelling approach to focus upon the nonlinear relationships of lead in ambient air, drinking water or diet, and blood lead. In Chamberlain's approach the nonlinearity to the uptake–

blood lead relationship is ascribed to a dose-dependent renal excretion rate for body lead. Nonlinear renal clearance of lead over a broad exposure range is not inconsistent with the plasma lead results of Manon and Cook (1984), DeSilva (1981), and Marcus (1985c), in that an increased renal excretion rate occurs with a proportionately increased lead fraction in plasma at higher exposure levels; i.e., all transfer coefficients are increased with elevated blood lead, as suggested by Chamberlain (1985).

In summary, both linear and nonlinear models have been applied to lead biokinetics under mainly steady-state conditions and mainly employing information from limited numbers of subjects. In many cases, depending upon the demands on the particular model, there may not be any added virtue of nonlinear over linear models, e.g., study of subjects in steady state at relatively low level of exposure. On the other hand, the various nonlinear relationships that are known to exist for external media/biological media relationships and further refined body compartments, e.g., plasma and erythrocyte pools for blood lead, require more complex approaches. The approaches of Marcus (1985c) and Chamberlain (1985) are particularly helpful in extending the use of modelling in rationalizing the various observed nonlinear relationships.

With the exception of the somewhat tenuous estimates of Harley and Kneip (1984) for biological half-times and tissue lead burdens of individuals from the ages of 1 to 20 years (Tables 1 and 2), few biokinetic models have focused on the developing organism, which is a major limitation, since young children are recognized as the key risk population for the adverse health effects of lead. Any attempt to produce precise models, however, is severely impeded by the highly labile nature of lead toxicokinetics in young children.

BIOLOGICAL MONITORING AND THE BIOKINETIC CHARACTERISTICS OF BIOLOGICAL INDICATORS FOR LEAD

Lead in blood

Blood lead (PbB) is the most commonly used biological indicator of both lead exposure and health risks in humans and experimental animals. It is also the indicator for which most data are available in terms of external versus internal exposure relationships, dose–effect and dose–population response relationships, etc.

Based on experimental data (Griffin *et al.*, 1975; Rabinowitz *et al.*, 1976; Chamberlain *et al.*, 1978) and epidemiological studies (O'Flaherty *et al.*, 1982; Kang *et al.*, 1983; Hryhorczuk *et al.*, 1985), PbB has been found to be a relatively dynamic and labile measure, and this biokinetic characteristic governs this indicator's merits and drawbacks in any exposure picture.

Blood lead reflects, biokinetically, both relatively recent lead exposure and the toxicologically active fraction of lead body burden in various soft tissues, at least under steady-state conditions or near steady state.

The degree to which PbB reflects the large body burden of lead sequestered in bone, and which has the potential to become toxicologically active, appears to depend on the subject's exposure status, age, and/or mineral metabolism. In one study of retired lead workers, for example, the level of lead in bone,

as determined *in vivo* by X-ray fluorescence spectrometry (Christoffersson *et al.*, 1984), was found to be strongly positively correlated with the PbB of the subjects, indicating that the primary determinant of PbB in these older individuals was the resorption of lead sequestered in bone. By contrast, workers still employed showed no correlation between PbB and the lead level in bone, indicating that current exposure was probably the main determinant of PbB in this group.

Manton (1985) demonstrated that lead isotope ratio data for the blood lead of two subjects followed for *ca.* 9 years was in accord with a contribution of *ca.* 70% of lead from bone to PbB. Also, the PbB elimination rate studies of O'Flaherty *et al.* (1982) and Hryhorczuk *et al.* (1985) show a dependence of elimination half-life on length of exposure time, a parameter directly related to bone lead burden.

The relative contribution of current uptake versus bone content of lead to PbB in young children, unlike the case for adults, is not well understood. The probability exists that lead resorption from bone to blood in children would be a more dynamic process than in adults, given the biological half-life of lead in bone of young children as estimated by Harley and Kneip (1984) and shown in Table 1 as being *ca.* 30% that of teenagers.

It is generally understood that PbB reflects a shorter exposure time than, say, lead in teeth, but it is not widely known just what this means in quantitative terms. Hence, it is of interest to examine the response of PbB with changes in exposure, particularly reduction in lead uptake as occurs when children grow older, and the relative stability of PbB as a function of time and/or development.

Available information on elimination rates for lead from blood to tissues and excreta consists of both experimental exposures under controlled conditions and surveys of PbB behaviour in human subjects with changes in exposure.

Using various experimental exposure methods, including isotopic tracer (Rabinowitz *et al.*, 1976; Chamberlain *et al.*, 1978) and chamber techniques (Griffin *et al.*, 1975), the biological half-life of PbB has been estimated as being on the order of 16–28 days, as depicted in Table 3.

The experimental results noted above mainly reflect the relatively fast component of what would appear to be a two-component PbB decay curve (O'Flaherty *et al.*, 1982; Kang *et al.*, 1983; Hryhorczuk *et al.*, 1985), since these studies were short in duration. It is therefore expected that an increase in the survey period and the number of sampling points (as well as biological differences) would be associated with considerable variability and increases

Table 3 Experimental studies of blood lead elimination rates in humans[a]

Conditions	*Half-life (days)*	*Reference*
1. Oral Pb-204, five adults	25	Rabinowitz *et al.*, 1976
2. Pb-203, all routes, 10 adults	16	Chamberlain *et al.*, 1978
3. Inhaled Pb aerosol, 18 adults, two doses 3.2 or 10.1 $\mu g/m^3$	28 (10.9 $\mu g/m^3$) 26 (3.2 $\mu g/m^3$)	Griffin *et al.*, 1975

in estimated half-lives for PbB. This appears to be the case in epidemiological surveys of PbB changes in human subjects having reduced exposure, as seen in Table 4.

Lead workers who were removed from active exposure for various reasons showed PbB decay half-lives that varied considerably: 20–130 days (O'Flaherty *et al.*, 1982); 79–133 days (estimated by the author from the elimination rate constants reported in the paper of Kang *et al.*, 1983); and a median of 619 days (Hryhorczuk *et al.*, 1985). Non-occupational subjects, who have also been studied in terms of alterations in lead exposure, had PbB half-life values on the order of 180–210 days (Thomas *et al.*, 1979; Delves *et al.*, 1984).

Data pertaining to the opposite process, the rate of PbB increase with increase in exposure, have been less well studied in human subjects since most of the available data have been derived from animal studies. However, from studies of newly employed lead workers (Tola *et al.*, 1973) and non-occupational volunteers (Griffin *et al.*, 1975) who inhaled metred lead aerosols in exposure chambers, it appears that an upward change in lead uptake leads to a plateau in higher PbB at *ca.* 60 days.

In summary, the PbB decay rates reported under experimental and epidemiological survey conditions indicate relatively short biological half-lives. The values of the half-lives vary with the type of study, e.g., length of survey and number of measurement points. For example, the lead-poisoned workers of Hryhorczuk *et al.* (1985) showed a median PbB decay half-life of 619 days when followed for more than 5 years. The PbB curves for these subjects probably included more of the slow decay component than in any of the other reports. With an increase in lead exposure, adults appear to require *ca.* 60 days to return to exposure steady state, i.e., a rise in the PbB curve followed by a plateau.

Turning to a related issue, the temporal stability of PbB over various intervals and differing exposure settings appears to differ in infants, children, and adults.

In infants, PbB is very unstable in the first year of life but increases in

Table 4 Epidemiological studies of blood lead elimination rates in humans

Study population	*Exposure conditions*	$T_{1/2}$ *(days)*	*Reference*
Lead workers, $n = 68$	Removed from exposure by work stoppage	20–130 (exposure-dependent)	O'Flahety *et al.*, 1982
Lead workers, $n = 77$, four smelters	Medical removal for elevated PbB	79–133[a]	Kang *et al.*, 1983
Workers with Pb poisoning $n = 65$	Medical removal with Pb intoxication	619 (median)	Hryhorczak *et al.*, 1985
Adult women	Pb in tapwater; Pb plumbing removed	180	Thomas *et al.*, 1979
Adult men	Oral exposure, reduced intake	180–120	Delves *et al.*, 1984

[a] Calculated from rate constants in report

stability during the second year (Rabinowitz *et al.*, 1984). As seen in Table 5, correlation among PbB levels at 6-month intervals is poor the first year, but the Spearman coefficients increase significantly in the second year, particularly from 18 to 24 months ($r = 0.61$). Furthermore, concordance as to the PbB category, in 6-month increments, showed that only 38% of the infants remained in their original exposure class (low, medium, or high) from birth to 24 months. The report of Winneke *et al.* (1985) supports the previous discussion in that children examined as to cord blood versus PbB 6–7 years later showed only modest correlation in the two measures ($r = 0.27$, $p < 0.05$).

Among older subjects, PbB stability over time is considerably greater, at least in terms of rank order. Figure 1 depicts a plot of 5-year follow-up PbB values for a group of children ($n = 50$) compared to their original concentrations, recorded when they were 10 months to 6.5 years of age (Schroeder *et al.*, 1985). A good correlation was obtained between the two measures ($r = 0.72$). Similarly, Lansdown *et al.* (1986) reported that the PbB values for 162 school-aged children drawn *ca.* 20 months apart showed a correlation of 0.52 between the two measures. In adults the temporal stability

Table 5 Spearman correlation coefficients for PbB at different ages (r)[a]

Age	*Birth*	*6 months*	*12 months*	*18 months*	*24 months*
Birth	—	0.10	0.20	0.09	0.19
6 mo	0.10	—	0.19	0.28	0.25
12 mo	0.20	0.19	—	0.41	0.36
18 mo	0.09	0.28	0.41	—	0.61
24 mo	0.19	0.25	0.36	0.61	—

[a] From Rabinowitz *et al.*, 1984, with permission

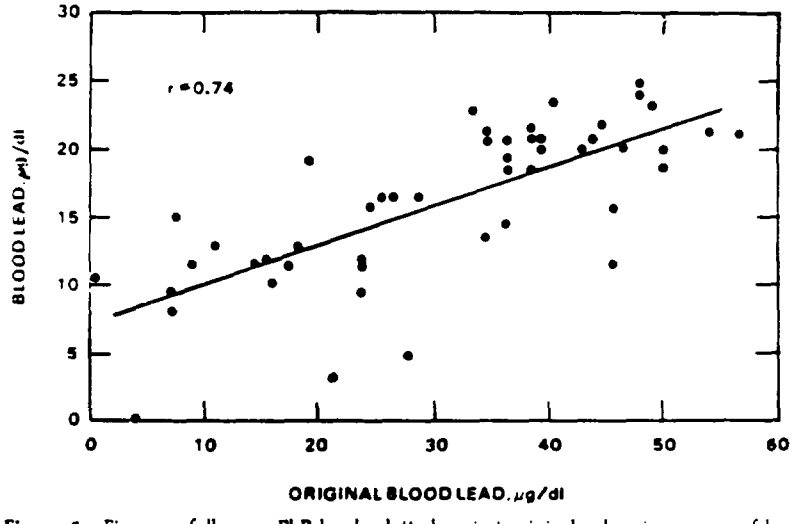

Figure 1 Five-year follow-up PbB levels plotted against original values in a group of lead-exposed children ($n = 50$). Adapted from Schroeder *et al.*, 1985

of PbB in 21 adults having relatively low environmental exposure was examined for periods of up to 11 months by Delves *et al.* (1984), and the variance of serial measurement was found to be less than 0.5 μg Pb/dl, where the male mean PbB was 12.2 and that of females was 8.5 μg Pb/dl. Hence, in the absence of major exposure changes, PbB values appear to be quite stable in adults.

In summary, the temporal stability of PbB is a function of age and stage of development. PbB values are most labile in infancy and tend to become more stable with age. In older children this stability is mainly in the form of preservation of rank order, whereas in adults there is also rather good preservation of absolute PbB values. These data suggest that single PbB measurements are least reliable in infancy but become more reliable with increasing age of the subject. The stability of the rank order in children as they get older may represent the relative level of body lead burden in bone. This would parallel what is seen in retired lead workers, where the main determinant of PbB is the bone lead burden (Hryhorczuk *et al.*, 1985).

Lead in teeth

The use of lead in mineralizing tissue, especially in teeth, as a biological indicator of lead exposure is based on the accumulation of the toxicant in these matrices as a function of both age and level of exposure. Hence, lead in teeth or bone provides a cumulative index of exposure over very extended time frames. Lead levels in shed teeth as an exposure indicator have been employed in a number of studies of the effects of lead on paediatric populations (e.g., Needleman *et al.*, 1979; Delves *et al.*, 1982; Ewers *et al.*, 1982; Grandjean *et al.*, 1984). Elevated levels of the element have been reported in whole teeth or their constituents as a function of poisoning history, point source proximity, or geographical location (Shapiro *et al.*, 1973; Needleman *et al.*, 1979; Steenhout and Pourtois, 1981; Ewers *et al.*, 1982; Delves *et al.*, 1982; Grandjean *et al.*, 1984).

The biokinetic aspects of lead deposition and relative distribution in dentition appear to be relatively complicated, and a number of factors governing the behaviour of lead in mineralizing tissue need to be recognized. The level of lead in whole teeth varies with the type of dentition, highest in incisors and decreasing to the premolars (e.g., Mackie *et al.*, 1977). In addition, the distribution of lead in tooth regions is heterogeneous and is variable in concentration stability over time, reflecting in part the developmental anatomy and physiology of dentition. The highest concentrations of lead are present in the inner and outer surfaces, i.e., the outer layer of enamel, and the circumpulpal dentine (Shapiro *et al.*, 1972, 1973; Brudevold *et al.*, 1977). Circumpulpal dentine is directly interfaced with the blood supply and provides the best index of lead accumulation as a function of systemic exposure. Enamel seems to remain relatively invariant in lead content, although one Finnish study of children with modest cumulative lead exposure suggests that enamel may adsorb lead from saliva in proportion to environmental exposure (Haavikko *et al.*, 1984).

The deposition of lead in primary dentine, the major component of dentine

on a mass basis (Al-Naimi *et al.*, 1980), remains unclear in terms of lead deposition rate, the time frame for lead deposition, and the mechanisms of deposition. Although lead in primary dentine is only *ca.* 10–30% that of circumpulpal dentine, this region accumulates lead with increased exposure (Shapiro *et al.*, 1973) and with postnatal age (Al-Naimi *et al.*, 1980). In the latter report, for example, primary dentine lead levels in young subjects up to 16 years of age, who lived in different areas of the United Kingdom, were significantly below corresponding values for individuals 40–72 years of age. In one subset of subjects the mean difference was *ca.* sevenfold. The mechanisms by which lead is postnatally deposited within the matrix of primary dentine is not clear. Carroll *et al.* (1972) have demonstrated, by electron microprobe techniques, that lead in mineralized dentine is not uniformly distributed but is laid down in interconnected pockets or channels with lead enrichment. These areas also correspond to regions of low mineralization. Hence, small amounts of lead may possibly move from circumpulpal to primary dentine along these lead-enriched channels.

Any detailed assessment of lead in dentition as a useful indicator is confounded by the fact that the various relevant studies have not employed a standard sampling protocol. Some researchers have employed whole tooth or crowns (Pinchin *et al.*, 1978; Steenhout and Pourtois, 1981; Delves *et al.*, 1982; Ewers *et al.*, 1982; Smith *et al.*, 1983), while others have employed lead levels in regions of teeth, generally secondary (circumpulpal) dentine (Shapiro *et al.*, 1973, 1975; Needleman *et al.*, 1979; Grandjean *et al.*, 1984). In most cases, lead levels in whole or dissected teeth have been used as such, but reporting of lead burden as a function of age has also been done.

Although marked differences in lead content across types of dentition have been recognized, there is also the question of how much variance exists in the measure within dentition type for tooth exfoliation in children, e.g., the two upper central incisors. Using central and lateral incisor crowns, Delves *et al.* (1982) noted that the extent to which lead values in central–central, lateral–lateral, or central–lateral pairs of shed incisors from a group of children exceeded relative analytical variance was 23, 35, and 54%, respectively. Subsequently, the same group found that the differences are maximized with differing jaw position, i.e., upper versus lower (Smith *et al.*, 1983). Variance within the jaw was considerably less. By contrast, variation within-tooth-type lead level appeared to be rather modest in the studies of Pinchin *et al.* (1978) and Ewers *et al.* (1982). However, a close comparison of these three studies is not readily made.

The relevance of the results of Delves *et al.* (1982) for some of the major surveys of the effects of lead exposure in children, employing tooth lead analysis, remains unclear. For example, in the study of Needleman *et al.* (1979), dentine zone analysis was carried out using concordance criteria for acceptability of replicate measurements. The relative impact of variation in lead level within tooth type may be increased at very low levels of concentration and decreased with higher concentration. There is no evidence to indicate that the relative biological variance of the type seen by Delves *et al.* persists with increasing concentration. As Delves *et al.* have acknowledged, their mean and median lead levels are lower than those noted elsewhere in

the United Kingdom, in Europe, and in the United States.

One key question is the degree to which lead levels in shed dentition correlate with the more common biological indicator, lead in blood. Since lead accumulates in teeth over a period of years (from formation through eruption until shedding), one might expect a moderate correlation with an indicator of more recent exposure such as PbB. Ewers *et al.* (1982) reported a correlation coefficient of 0.47 between PbB and incisor crowns in a group of 83 children. Similarly, in the report of Smith *et al.* (1983), a correlation coefficient of 0.50 was obtained for 92 children across all teeth analysed. Further analysis as a function of jaw position (lower or upper incisors) or exposure category produced a value of 0.58 for lower teeth. Correlations by exposure group produced more widely ranging values, from −0.09 for medium lead exposure to a value of 0.43 for the high-lead group.

In summary, the use of shed dentition as a biological indicator of cumulative exposure to lead in children would appear to be appropriate under certain conditions. These conditions include rigorous steps to minimize variance in the measure: multiple tooth sampling restricted to the same type (and location if possible), or use of concordance criteria for acceptance or rejection of lead levels in replicate sampling. By its nature, measurement of lead in teeth is a retrospective index of exposure to lead, and this measure is not as inherently useful for regulatory policy or clinical intervention/management of lead exposure and intoxication as is PbB. The various prospective studies currently under way in different countries for lead exposure/effects in children include some that utilize serial measurement of PbB in the paediatric subjects as they develop. Comparison of these multiple measurements with lead in shed dentition in the future would be valuable in establishing blood lead–tooth lead relationships.

Chelatable lead

Chelatable lead refers to that fraction of the body lead burden that is mobilized into urine by a single dose of the chelating agent $Ca\text{-}Na_2$ EDTA, with the dose standardized as to body surface or weight of the subject. It is an exposure monitoring term operationally distinct from chelation therapy, a clinical procedure employed to reduce the toxicological capacity of a given lead exposure. Since the EDTA challenge test is an invasive procedure and one having other constraints (Piomelli *et al.*, 1984), it is only employed when elevated body lead burden has been established by other means (PbB and erythrocyte zinc protoporphyrin).

Evidence of lead intoxication is taken as the ratio of urinary lead excreted over 8 h to milligrams of chelant in excess of 0.60. With a ratio of 0.60–0.69, either further monitoring or treatment is carried out, depending on the child's age. A ratio of 0.70 or higher dictates a course of chelation therapy in all cases (Piomelli *et al.*, 1984).

Chelatable lead is widely viewed as the most useful index of toxicologically active lead burden in adults and children (US Centers for Disease Control, 1985; World Health Organization, 1977), and it is of interest to consider this

exposure indicator compared to PbB in childhood lead toxicity.

Chelatable lead is widely accepted as representing removal from soft tissue (e.g., Chisolm and Barltrop, 1979), but some mobilizable compartment for lead storage in bone must also be providing a sizeable contribution. Evidence for the bone source includes (1) the age dependency of chelatable lead in non-occupationally exposed subjects, whereas lead in soft tissue is rather invariant with age (Araki, 1973; Araki and Ushio, 1982); (2) experimental animal (Hammond, 1971, 1973) and *in vitro* bone culture data (Rosen and Markowitz, 1980) showing removal of lead from bone; and (3) the tracer modelling data of Rabinowitz *et al.* (1977), which define a bone compartment for lead which is kinetically well mixed with those for blood and soft tissue.

Given the above indications that a sizeable fraction of mobilizable lead exists in bone, and that this portion of body burden is indexed by chelatable lead testing, the question arises as to exactly how chelatable lead is related to PbB in assessment of overall systemic exposure risk. In the detailed report of Piomelli and co-workers (1984) describing the management of childhood lead intoxication, a survey of 210 children from four lead poisoning centres in the United States in terms of chelatable lead versus PbB, suggests that PbB may indeed understate toxicity risk level. Table 6 notes the percentages of these children whose chelatable lead ratios exceed 0.60, a ratio showing lead intoxication, as a function of different PbB values. As can be seen, PbB levels below 30 μg/dl are not associated with worrisome mobilizable lead levels, while at somewhat higher PbB values the percentage of such children is significant and increases considerably with PbB. These data also provide an additional argument for viewing PbB values below 30 μg/dl as the upper limit of permissible exposure (US Centers for Disease Control, 1985).

Table 6 Percentage of children exceeding EDTA challenge ratio as a function of PbB (n = 210)[a]

PbB (μg/dl)	*Percentage exceeding*
< 30	0
30–39	11.5
40–49	37.9
50–59	49.2

[a] From Piomelli *et al.*, 1984

Lead in hair

In theory, lead in hair would appear to be an ideal biological indicator. The sampling is noninvasive, the medium is indefinitely stable in storage, and a temporal profile of exposure along the hair length is available. Hair has been used in a number of surveys of lead exposure in children (e.g., Marlow and Errera, 1982; Thatcher *et al.*, 1982).

In practical terms, however, there are some severe limits on the use of hair lead as a systemic or internal indicator of exposure, one of which is methodological and the other metabolic. With hair, it is virtually impossible

to avoid external contamination by ubiquitous lead, and there are no accurate validation techniques for assessing hair cleaning techniques. This is not to say that hair cannot be used as an external indicator, where it would still be expected to show some correlation with various health end-points. For example, fallout of particulate lead onto hair surface or uptake of lead from bathing water would reflect air lead and water lead. The question here, however, is usefulness as an internal indicator. In addition to methodological hazards, the biokinetics of lead in hair is not understood to the extent required for its reliable use as a biological indicator. This is especially true at low levels of exposure associated with subtle effects in children.

Lead in bone

Lead accumulates in the trabecular and cortical bone as a function of both age and exposure. It represents the major repository of lead in the human body, accounting for at least 95% of total body burden. While the overall biokinetics of lead in bone suggest a compartment with a long biological half-life, of the order of a decade or so, some fraction of this amount can be remobilized via various bone resorption processes as noted earlier in this report.

The direct use of bone lead levels as a biological indicator was not possible in the past, and much of our information on lead in this matrix has involved autopsy sampling. More recently, however, a number of laboratories have been occupied with the development and use of *in vivo* methods for measurement of lead in bone. For example, Christoffersson *et al.* (1984) have developed an X-ray fluorescence technique for the measurement of lead in long bones, and have applied the method to lead exposure status of both active and retired lead workers. Similarly, *in vivo* X-ray fluorescence techniques are being developed for assessment of lead in the long bones of children (Wielpolski *et al.*, 1983).

Until the newly developed *in vivo* methods described above can be applied in relatively large-scale survey schemes, it is not possible to say what the practical merits of such an approach would be in the biological montoring of lead exposure. Certainly, the tandem use of *in vivo* bone lead measurement with serial PbB sampling would provide a potent measure of both circulating, biologically active lead and simultaneously, potentially toxic, mobilizable toxicant.

USE OF BIOLOGICAL EFFECT INDICATORS IN THE MONITORING OF LEAD EXPOSURE

When referring to the early or 'subcritical' effects of lead in humans as biological indicators of exposure, the primary concern is the alterations in those levels of intermediates in the haem biosynthesis pathway that are known to be affected by the presence of lead. Three processes that have lent themselves to examination in the context of biological monitoring include: (1) the inhibition of delta-aminolaevulinic acid dehydratase (δ-ALA-D);

(2) the accumulation of delta-aminolaevulinate in urine (δ-ALA-U) due to inhibition of the δ-ALA dehydratase enzyme and the feedback-mediated derepression of delta-aminolaevulinate synthetase enzyme; and (3) the accumulation of zinc protoporphyrin (ZPP) in erythrocytes owing to inhibited action of ferrochelatase or iron transport to the iron-insertion site. Detailed discussions of these effects can be found in the documents of the US Environmental Protection Agency (1977) and the World Health Organization (1977).

Early effect indicators, whatever their limitations in a preventive context of lead exposure, have the virtue of indicating that fraction of measurable lead that is actually biologically active. Also, given the widespread use of measurement of erythrocyte protoporphyrin prior to actual PbB determinations in the large-scale screening of children, these effect indicators will remain on the scene.

Employed as an index of lead exposure, the inhibition of δ-ALA-D in erythrocytes would appear to offer little advantage over direct measurement of PbB. In erythrocytes the enzyme is vestigial, and its inhibition requires the presence of lead ion interacting with the sulphydryl group in the proximity of the active site (Mitchell *et al.*, 1977). In addition, a number of methodological problems abound that would further serve to minimize this measure's attraction as a substitute for PbB.

On a group basis, the elevation of ALA level in the urine of children and adults is taken as an effect indicator of lead exposure. The effect becomes most pronounced above a 'threshold' of *ca.* 40 μg/dl PbB, and the relationship of the measure to PbB below this value is clouded in some disagreement (USEPA, 1977). Since much of the current interest in lead exposure of children involves PbB values below 40 μg/dl, δ-ALA-U may not be sensitive enough to be of much use. There are also some methodological limitations, including the desirability of obtaining 24-h urine samples.

At present, the most popular biological effect indicator of lead exposure is erythrocyte ZPP. Elevation of ZPP is a sensitive indicator, showing a threshold of response in children of *ca.* 15 μg/dl PbB (Piomelli *et al.*, 1982; Hammond *et al.*, 1985) and shows a tight correlation with PbB (log-transformed ZPP data). ZPP elevation occurs also in the presence of iron deficiency, a common occurrence in young children, and any use of this measure for exposure monitoring would require correction for, and determination of, the level of iron deficiency. In older children, ZPP levels are a cleaner measure.

Elevation of erythrocyte ZPP lags any increases in PbB due to increased exposure. ZPP levels, furthermore, remain elevated when exposure has ceased. The former arises from ZPP insertion into cells occurring only during active intoxication of bone marrow, whereas ZPP decay, when exposure ceases, is governed to some degree by the rate of erythrocyte turnover.

Various studies have been directed to the relative merits of ZPP versus other indicators in studies of effect outcomes. These have produced something of a mixed picture (Fischbein *et al.*, 1980; Hammond *et al.*, 1980; Saenger *et al.*, 1982). For example, Fischbein and co-workers (1980) reported that ZPP was elevated in those workers showing central nervous system or gastrointestinal symptoms. By contrast, only 5% of these workers had PbB

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levels in excess of 40 μg/dl. Hammond *et al.* (1980) used ZPP, δ-ALA-U, and PbB as exposure indicators, and found that PbB was not particularly advantageous in predicting subjective neurological symptoms compared to the haematological effect indicators.

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